

U.S. Patent Appl. No. 09/963,521—Ziegler et al.

I. AMENDMENTS TO THE CLAIMS

1-18. (Cancel)

19. (Previously Presented) A process for the preparation of L-threonine comprising fermenting L-threonine producing *Corynebacterium* or *Brevibacterium* in which the *Corynebacterium glutamicum thrE* gene encoding a threonine export protein is overexpressed by increasing the copy number of said gene, and isolating said L-threonine produced by said *Corynebacterium*.

20. (Currently Amended) The process of claim 19, wherein ~~said *Corynebacterium* or *Brevibacterium* also are overexpressed by increasing the copy number of the~~ *Corynebacterium glutamicum pyc* gene encoding pyruvate carboxylase is also overexpressed in said *Corynebacterium* or *Brevibacterium* by increasing the copy number of said gene.

21. (Currently Amended) The process of claim 19, wherein ~~said *Corynebacterium* or *Brevibacterium* also are overexpressed by increasing the copy number of the~~ *Corynebacterium glutamicum hom* gene encoding for homoserine dehydrogenase is also overexpressed in said *Corynebacterium* or *Brevibacterium* by increasing the copy number of said gene.

22. (Currently Amended) The process of claim 19, wherein ~~said *Corynebacterium* or *Brevibacterium* also are overexpressed by increasing the copy number of the~~ *Corynebacterium glutamicum hom^{dr}* allele encoding a feedback-resistant homoserine dehydrogenase is also overexpressed in said *Corynebacterium* or *Brevibacterium* by increasing the copy number of said allele.

23. (Currently Amended) The process of claim 19, wherein ~~said *Corynebacterium* or *Brevibacterium* also are overexpressed by increasing the copy number of the~~ *Corynebacterium glutamicum mqo* gene encoding malate:quinone oxidoreductase is also overexpressed in said *Corynebacterium* or *Brevibacterium* by increasing the copy number of said gene.

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24. (Currently Amended) The process of claim 19, wherein the *Corynebacterium* of the species *Corynebacterium glutamicum* are used.

25. (Currently Amended) The process of claim 19, wherein the *Brevibacterium* of the species *Brevibacterium flavum* are used.

26. (Currently Amended) A process for the preparation of L-threonine comprising fermenting L-threonine producing coryneform bacteria in which the *Corynebacterium glutamicum* thrE gene encoding a threonine export carrier protein is overexpressed by increasing the copy number of the thrE gene, and isolating said L-threonine produced by said coryneform bacteria, wherein said coryneform bacteria have been transformed with a plasmid vector comprising the *C. glutamicum* thrE gene encoding said threonine export carrier protein and said plasmid vector is pZ1thrE, which is deposited in *Brevibacterium flavum* under deposit number DSM12840 (support page 12, line 23, 24).

27. (Currently Amended) The process of claim 26, wherein ~~said coryneform bacteria also overexpress by increasing the copy number of the~~ *Corynebacterium glutamicum* pyc gene encoding pyruvate carboxylase is also overexpressed in said coryneform bacteria by increasing the copy number of said gene.

28. (Currently Amended) The process of claim 26, wherein ~~said coryneform bacteria also overexpress by increasing the copy number of the~~ *Corynebacterium glutamicum* hom gene encoding for homoserine dehydrogenase is also overexpressed in said coryneform bacteria by increasing the copy number of said gene.

29. (Currently Amended) The process of claim 26, wherein ~~said coryneform bacteria also overexpress by increasing the copy number of the~~ *Corynebacterium glutamicum* hom^{dr} allele encoding a feedback-resistant homoserine dehydrogenase is also overexpressed in said *Corynebacterium* or *Brevibacterium* by increasing the copy number of said allele.

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30. (Currently Amended) The process of claim 26, wherein ~~said coryneform bacteria also overexpress by increasing the copy number of the~~ *Corynebacterium glutamicum* *mgo* gene encoding malate:quinone oxidoreductase is also overexpressed in said coryneform bacteria by increasing the copy number of said gene.

31. (Currently Amended) The process of claim 26, wherein ~~the~~ coryneform bacteria of the genus *Corynebacterium* are used.

32. (Currently Amended) The process of claim 31, wherein ~~the~~ *Corynebacterium* of the species *Corynebacterium glutamicum* are used.

33. (Currently Amended) The process of claim 26, wherein the coryneform bacteria of the genus *Brevibacterium* are used.

34. (Currently Amended) The process of claim 33, wherein the *Brevibacterium* of the species *Brevibacterium flavum* are used.

35. (Currently Amended) A process for the fermentative preparation of L-threonine comprising:

- (a) fermenting L-threonine producing *Corynebacterium* or *Brevibacterium* bacteria in which a *C. glutamicum* *thrE* gene encoding a threonine export carrier protein is overexpressed by increasing the copy number of said gene; and, wherein ~~said coryneform bacteria also overexpress by increasing the copy number~~ one or more of the coryneform genes selected from the group consisting of: the *Corynebacterium glutamicum* *pyc* gene encoding pyruvate carboxylase, the *Corynebacterium glutamicum* *hom* gene encoding for homoserine dehydrogenase, the *Corynebacterium glutamicum* *hom^{dr}* allele encoding a feedback-resistant homoserine dehydrogenase, and the *Corynebacterium glutamicum* *mgo* gene encoding for malate:quinone

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oxidoreductase are overexpressed by increasing the copy number of said genes;

- (b) concentrating the L-threonine in the fermentation medium or in said coryneform bacteria; and
- (c) isolating L-threonine from the fermentation medium or coryneform bacteria of step (b).

36. (Currently Amended) A process for the fermentative preparation of L-threonine comprising:

- (a) fermenting L-threonine producing coryneform bacteria in which a thrE gene encoding a threonine export carrier protein is overexpressed by increasing the copy number of the gene; and, wherein said ~~coryneform bacteria also overexpress by increasing the copy number of one or more of the coryneform genes selected from the group consisting of:~~ the Corynebacterium glutamicum pyc gene encoding pyruvate carboxylase, the Corynebacterium glutamicum hom gene encoding for homoserine dehydrogenase, the Corynebacterium glutamicum hom^{dr} allele encoding a feedback-resistant homoserine dehydrogenase, and the Corynebacterium glutamicum mqo gene encoding for malate:quinone oxidoreductase are overexpressed by increasing the copy number of said genes;
- (b) concentrating the L-threonine in the fermentation medium or in said coryneform bacteria; and
- (c) isolating L-threonine from the fermentation medium or coryneform bacteria of step (b)

wherein said coryneform bacteria have been transformed with a plasmid vector comprising the C. glutamicum thrE gene encoding said threonine export carrier protein and said plasmid vector is pZ1thrE, which is deposited in Brevibacterium flavum under deposit number DSM12840.

37. (Previously Presented) The process of claim 19, wherein said thrE gene comprises a polynucleotide encoding a protein comprising the amino acid sequence of SEQ ID NO: 2.

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38. (Previously Presented) The process of claim 37, wherein said polynucleotide comprises nucleotides 398 to 1864 of SEQ ID NO: 1.

39. (Currently Amended) The process of claim 38, wherein said thrE gene comprises SEQ ID NO: 1 ~~and or~~ SEQ ID NO: 3.

40. (Previously Presented) A process for the preparation of L-threonine comprising fermenting L-threonine producing *Corynebacterium* or *Brevibacterium* bacteria in which the *Corynebacterium glutamicum* thrE gene encoding a threonine export protein is overexpressed by operatively linking said gene to a promoter, and isolating said L-threonine produced by said *Corynebacterium*.

41. (Currently Amended) The process of claim 40, wherein ~~said *Corynebacterium* or *Brevibacterium* also are overexpressed by operatively linking the *Corynebacterium glutamicum* pyc gene encoding pyruvate carboxylase~~ is also overexpressed in said *Corynebacterium* or *Brevibacterium* by operatively linking said gene to a promoter.

42. (Currently Amended) The process of claim 40, wherein ~~said *Corynebacterium* or *Brevibacterium* also are overexpressed by operatively linking the *Corynebacterium glutamicum* hom gene encoding for homoserine dehydrogenase~~ is also overexpressed in said *Corynebacterium* or *Brevibacterium* by operatively linking said gene to a promoter.

43. (Currently Amended) The process of claim 40, wherein ~~said *Corynebacterium* or *Brevibacterium* also are overexpressed by operatively linking the *Corynebacterium glutamicum* hom^{dr} allele encoding a feedback-resistant homoserine dehydrogenase~~ is also overexpressed in said *Corynebacterium* or *Brevibacterium* by operatively linking said allele to a promoter.

44. (Currently Amended) The process of claim 40, wherein ~~said *Corynebacterium* or *Brevibacterium* also are overexpressed by operatively linking the *Corynebacterium glutamicum* mqo gene encoding malate:quinone oxidoreductase~~ is also overexpressed in said *Corynebacterium* or *Brevibacterium* by operatively linking said gene to a promoter.

45. (Currently Amended) The process of claim 40, wherein ~~the~~ Corynebacterium bacteria of the species Corynebacterium glutamicum are used.

46. (Currently Amended) The process of claim 40, wherein ~~the~~ Brevibacterium bacteria of the species Brevibacterium flavum are used.

47. (Currently Amended) A process for the preparation of L-threonine comprising fermenting L-threonine producing coryneform bacteria in which the Corynebacterium glutamicum thrE gene encoding a threonine export carrier protein is overexpressed by operatively linking said thrE gene to a promoter, and isolating said L-threonine produced by said coryneform bacteria, wherein said coryneform bacteria have been transformed with a plasmid vector comprising the C. glutamicum thrE gene encoding said threonine export carrier protein and said plasmid vector is pZ1thrE, which is deposited in Brevibacterium flavum under deposit number DSM12840 (~~support page 12, line 23, 24~~).

48. (Currently Amended) The process of claim 47, wherein ~~said coryneform bacteria also overexpress by operatively linking~~ the Corynebacterium glutamicum pyc gene encoding pyruvate carboxylase is also overexpressed in said coryneform bacteria by operatively linking said gene to a promoter.

49. (Currently Amended) The process of claim 47, wherein ~~said coryneform bacteria also overexpress by operatively linking~~ the Corynebacterium glutamicum hom gene encoding for homoserine dehydrogenase is also overexpressed in said coryneform bacteria by operatively linking said gene to a promoter.

50. (Previously Presented) The process of claim 47, wherein said coryneform bacteria also overexpress by operatively linking the Corynebacterium glutamicum hom^{dr} allele encoding a feedback-resistant homoserine dehydrogenase to a promoter.

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51. (Currently Amended) The process of claim 47, wherein ~~said coryneform bacteria also overexpress by operatively linking~~ the *Corynebacterium glutamicum* mqo gene encoding malate:quinone oxidoreductase is also overexpressed in said coryneform bacteria by operatively linking said gene to a promoter.

52. (Currently Amended) The process of claim 47, wherein the coryneform bacteria of the genus *Corynebacterium* are used.

53. (Currently Amended) The process of claim 52, wherein ~~the~~ *Corynebacterium* of the species *Corynebacterium glutamicum* are used.

54. (Currently Amended) The process of claim 47, wherein ~~the~~ coryneform bacteria of the genus *Brevibacterium* are used.

55. (Currently Amended) The process of claim 54, wherein ~~the~~ *Brevibacterium* of the species *Brevibacterium flavum* are used.

56. (Currently Amended) A process for the fermentative preparation of L-threonine comprising:

- (a) fermenting L-threonine producing *Corynebacterium* or *Brevibacterium* bacteria in which a *C. glutamicum* thrE gene encoding a threonine export carrier protein is overexpressed by operatively linking said gene to a promoter; and, wherein said coryneform bacteria also overexpress by operatively linking one or more of the coryneform genes selected from the group consisting of: the *Corynebacterium glutamicum* pyc gene encoding pyruvate carboxylase, the *Corynebacterium glutamicum* hom gene encoding for homoserine dehydrogenase, the *Corynebacterium glutamicum* hom^{dr} allele encoding a feedback-resistant homoserine dehydrogenase, and the *Corynebacterium glutamicum* mqo gene encoding for malate:quinone oxidoreductase are overexpressed by operatively linking said genes to a promoter;

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- (b) concentrating the L-threonine in the fermentation medium or in said coryneform bacteria; and
- (c) isolating L-threonine from the fermentation medium or coryneform bacteria of step (b).

57. (Currently Amended) A process for the fermentative preparation of L-threonine comprising:

- (a) fermenting L-threonine producing coryneform bacteria in which a thrE gene encoding a threonine export carrier protein is overexpressed by operatively linking said gene to a promoter; and, wherein said ~~coryneform bacteria also overexpress by operatively linking~~ one or more of the coryneform genes selected from the group consisting of: the Corynebacterium glutamicum pyc gene encoding pyruvate carboxylase, the Corynebacterium glutamicum hom gene encoding for homoserine dehydrogenase, the Corynebacterium glutamicum hom^{dr} allele encoding a feedback-resistant homoserine dehydrogenase, and the Corynebacterium glutamicum mqo gene encoding for malate:quinone oxidoreductase are overexpressed by operatively linking said genes to a promoter;
- (b) concentrating the L-threonine in the fermentation medium or in said coryneform bacteria; and
- (c) isolating L-threonine from the fermentation medium or coryneform bacteria of step (b)

wherein said coryneform bacteria have been transformed with a plasmid vector comprising the C. glutamicum thrE gene encoding said threonine export carrier protein and said plasmid vector is pZ1thrE, which is deposited in Brevibacterium flavum under deposit number DSM12840.

58. (Previously Presented) The process of claim 40, wherein said thrE gene comprises a polynucleotide encoding a protein comprising the amino acid sequence of SEQ ID NO: 2.

59. (Previously Presented) The process of claim 58, wherein said polynucleotide comprises nucleotides 398 to 1864 of SEQ ID NO: 1.

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60. (Currently Amended) The process of claim 59 , wherein said thrE gene comprises SEQ ID NO: 1 and or SEQ ID NO: 3.